

CHLORINATION STUDIES. IV¹. THE REACTION OF AQUEOUS HYPOCHLOROUS
ACID WITH PYRIMIDINE AND PURINE BASES.

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Received July 12, 1973

SUMMARY

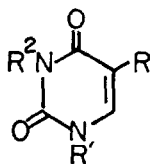
The labile intermediates and stable end products formed by the reaction of aqueous HOCl with thymine, uracil, 5-Br-uracil, N,N-dimethyluracil and 6-methyluracil have been identified. The purine ring system of guanine, adenine and xanthine was more resistant to attack by aqueous HOCl and reaction times of one week resulted in the formation of parabanic acid. Caffeine and theophylline under similar reaction conditions yielded N,N-dimethylparabanic acid.

Our interest in the chemical transformations by aqueous "hypochlorous acid"² of the pyrimidine and purine bases of nucleic acids arose from the known³ lethal action of low concentrations of HOCl on bacteria. The extent to which lethal actions result from modification, or destruction, of microbial DNA is not yet clear. The behavior of isolated bases may help in the analysis of more complex systems. We recently described⁴ the chemical modification of cytosine by aqueous HOCl, but little is known of the labile intermediates (which might themselves be chlorinating agents within the cell) formed by the reaction of HOCl with other nucleic acid bases (however, cf⁵).

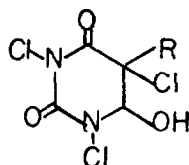
Pyrimidine Bases. (a) Thymine (I) and 5-Br-Uracil (II). Reaction of either I or II for 5 minutes with 2 gram equivalents of aqueous HOCl afforded a precipitate (III and IV respectively) together with the water soluble products V and VI. The quantity of precipitate, relative to V and VI,

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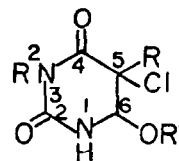
decreased after a reaction time of 1 hour. Hot water converted III and V to the same dichloro-compound believed to be either VII or the isomeric structure with the N-chloro group at N-1.



- I, $R=CH_3$, $R^1=R^2=H$
 II, $R=Br$, $R^1=R^2=H$
 III, $R=CH_3$, $R^1=R^2=Cl$
 IV, $R=Br$, $R^1=R^2=Cl$



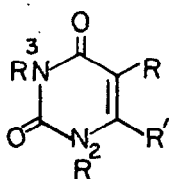
- V, $R=CH_3$
 VI, $R=Br$



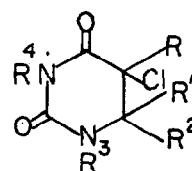
- VII, $R=CH_3$, $R^1=H$, $R^2=Cl$
 VIII, $R=CH_3$, $R^1=C_2H_5$, $R^2=H$

(a) Uracil (IX) and 5-Cl-Uracil (X). Reaction of

uracil (IX) with 2 gram equivalents of HOCl produced a precipitate (X) and a water soluble component (XI). 5-Cl-Uracil (X) when reacted with 4 gram equivalents of HOCl also produced a trichloro product (XI) (Mol. ion at m/e 214, 216, 218 and 220), and UV absorption at 275 nm was in agreement with this structural assignment. A second compound (probably XII) showing only end absorption in the UV proved too labile to isolate pure. When dissolved in water XI and XII yielded 5-Cl uracil (X).



- IX, $R=R^1=R^2=R^3=H$
 X, $R=Cl$, $R^1=R^2=R^3=H$
 XI, $R=R^2=R^3=Cl$, $R^1=H$
 XIII, $R=R^2=R^3=H$, $R^1=CH_3$
 XIV, $R=R^1=H$, $R^2=R^3=CH_3$
 XIIIa, $R=Cl$, $R^2=R^3=H$, $R^1=CH_3$
 XIVa, $R=Cl$, $R^1=H$, $R^2=R^3=CH_3$



- XII, $R=R^3=R^4=Cl$, $R^1=OH$, $R^2=H$
 XV, $R=Cl$, $R^1=CH_3$, $R^2=OH$, $R^3=R^4=H$
 XVI, $R=Cl$, $R^1=H$, $R^2=OH$, $R^3=R^4=CH_3$
 XVII, $R=R^1=H$, $R^2=OH$, $R^3=R^4=CH_3$

(c) 6-Methyl Uracil (XIII) and N,N-Dimethyl Uracil (XIV).

Reaction of either XIII or XIV with 2 gram equivalents of HOCl afforded the corresponding 5,5-dichloro-6-hydroxy-5,6-dihydrouracil derivatives XV and

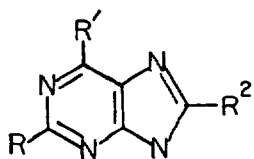
XVI (the latter product was contaminated with the monochlorohydrin (XVII)).

Water converted XV and XVI+XVII to the 5- Cl compounds XIIIa, and XIVa.

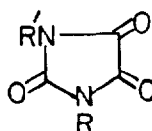
Purine Bases. (a) Guanine (XVIII), Adenine (XIX) and Xanthine (XX).

Reaction times of 1 or 2 hours with 2 gram equivalents of HOCl resulted in the recovery (>90%) of starting material. However, after a reaction time of 1 week parabanic acid (XXI) was recovered in yields of 90, 20 and 50% respectively. Parabanic acid is a known product from the electrolytic oxidation of uric acid (XXII),⁷ γ -irradiation of xanthine (XX)⁸ and laser irradiation of a mixture of guanine and methylene blue.⁹ Uracil (IX) also yields XXI on photolysis.¹⁰

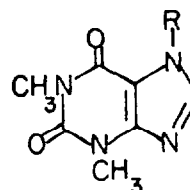
(b) Caffeine (XXIII) and Theophylline (XXIV). After a 1 week reaction time these compounds were converted (each in 90% yield) to N,N-dimethyl parabanic acid (XXV).



XVIII, R=NH₂, R¹=OH, R²=H
 XIX, R=R²=H, R¹=NH₂
 XX, R=R¹=OH, R²=H
 XXII, R=R¹=R²=OH



XXI, R=R¹=H
 XXV, R=R¹=CH₃



XXIII, R=CH₃
 XXIV, R=H

EXPERIMENTAL

Aqueous solutions of HOCl were prepared by the method¹¹ of Higuchi and Hasegawa. Mass spectra (70 eV) were recorded with a Finnigan 1015 Quadrupole instrument. Chlorination reactions were conducted at room temperature by dropwise addition of HOCl, with continuous stirring, to the substrate (1 mmole) dissolved or suspended in water (5 ml). Reaction products were examined by TLC (cellulose MN 300 CM in 70% aqueous methanol) and spots were visualized by chlorox spray or starch-potassium iodide spray.

Reaction of Thymine (I) with HOCl. After 5 minutes reaction with 2 g

equivs of HOCl, III precipitated, m.p. 270-290° (decomp). Found: C, 30.59; H, 2.34; N, 14.42; Cl, 36.1. $C_5H_4N_2O_2Cl_2$ requires: 30.79; H, 2.06, N, 14.36; Cl, 36.4%. Mass spectrum: M^+ m/e 194, 196, 198. Lyophilization of the filtrate yielded V. Mass spectrum M^+ m/e 248, 250, 252 and 254. Recrystallization of III or V from water yielded VII, m.p. 164-6°. Found: C, 28.04; H, 2.81; N, 13.55; Cl, 32.8; $C_5H_6N_2O_3Cl_2$ requires: C, 28.19; H, 2.83; N, 13.15; Cl, 33.3%. Recrystallization of III from ethanol gave VIII, m.p. 218-9°. Found: C, 40.36; H, 5.17; N, 13.56, Cl, 17.4. $C_7H_{11}N_2O_3Cl$ requires: C, 40.65; H, 5.36; N, 13.55; Cl, 17.2%.

Reaction of II with HOCl. Reaction for 5 mins with 2 g equivs of HOCl gave a precipitate, IV. Found: C, 17.97; H, 0.75; N, 10.84; Cl, 26.6; Br, 29.9. $C_4HN_2O_3Cl_2Br$ requires: C, 18.48; H, 0.38; N, 10.77; Cl, 27.3; Br, 30.7%. Lyophilization of the filtrate yielded VI. Mass spectrum (M minus $ClN=CO$)⁺ at m/e 233, 235, 237, 239.

Reaction of IX and X with HOCl. 5-Chlorouracil (X) m.p. 320° (lit.¹² m.p. 324-5°) precipitated from the reaction of 2 g equivs of HOCl with IX. Lyophilization of the filtrate yielded XI which also precipitated from the action of 4 g equivs of HOCl with X. Found: C, 20.57; H, 1.14; N, 11.97; Cl, 44.6. $C_4HN_2O_2Cl_3 \cdot H_2O$ requires: C, 20.57; H, 1.29; N, 12.00; Cl, 45.5%. Mass spectrum: M^+ m/e 214, 216, 218, 220. Lyophilization of the filtrate gave XII, UV_{max} 275 nm. Water converted XI and XII to 5-chlorouracil (X).

Reaction of XIII and XIV with HOCl. Treatment of XIII with 1 g equiv of HOCl yielded 5-chloro-6-methyl uracil (XIII, R=Cl), m.p. >300°. Mass spectrum: M^+ m/e 160, 162. 2 g equivs of HOCl afforded XV, m.p. 275-280° (lit.⁶ m.p. 275-7°). Mass spectrum: M^+ absent, (M minus NCO)⁺ at m/e 170, 172, 174. N,N-Dimethyl uracil (XIV) and 2 g equivs of HOCl yielded XVI. Mass spectrum: M^+ m/e 226, 228 plus XVII (M^+ m/e 192, 194). Water converted XV and (XVI + XVII) to XIII (R=Cl) respectively.

Reaction of Purines with 2 g Equivs of HOCl. After 7 days reaction the filtrate from the reaction of guanine (XVIII) was lyophilized. Chromatogra-

phy on MN-2100-CM cellulose and methanol:water (7:3) eluted parabanic acid (XXI), m.p. 238-240° (lit.¹³ m.p. 241-3° decomp). Yield 90%. Adenine (XIX) and xanthine (XX) yielded parabanic acid (20 and 50% respectively); identical mass spectrum with authentic material.¹³ Caffeine (XXIII) and theophylline (XXIV) yielded N,N-dimethylparabanic acid (90%) m.p. 151-3° (lit.¹⁴ m.p. 152-3°) whose mass spectrum was identical with authentic material.

Acknowledgment: This research was funded by the Planetary Program Office, Office of Space Science, NASA Headquarters under grant NGR-05-020-004 (J. Lederberg, Principal Investigator).

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